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Lack of hepatotoxic interaction between the anticonvulsant drugs phenytoin, sodium valproate and phenobarbital in the rat

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Phenytoin and valproic acid are widely used anticonvulsant drugs that have been implicated in rare cases of idiosyncratic hepatotoxicity in human beings [1–3]. Liver damage attributed to phenytoin usually occurs in the context of a hypersensitivity (allergic) reaction [1, 2], whereas the hepatotoxicity caused by valproate is believed to result from a metabolic idiosyncrasy in susceptible individuals [2, 3]. Anticonvulsant-induced hepatotoxicity frequently occurs in epileptic patients taking two or more anticonvulsant drugs [2]. Indeed, polytherapy-induced interactions have been suggested to be a major cause of many types of chronic toxicity associated with anticonvulsant drug therapy, and this has resulted in an advocacy for monotherapy [4]. However, control of seizures with a single drug is not always possible and, therefore, a knowledge of potential interactions is essential to devise therapeutic regimens which minimize the risk of adverse reactions. We have set up potential interactions between phenytoin, sodium valproate and phenobarbital, at doses approximately ten times human therapeutic levels, as a possible cause of hepatotoxicity in rats and thus a model for liver damage that can occur in human beings.

Materials and methods

Forty male albino Wistar rats (200 g, Charles River, Canada Inc.) were divided into ten treatment groups. Water and Purina Certified Rodent Chow No. 5002 were supplied *ad lib.* Anticonvulsant drugs, as their sodium salts, valproic acid (300 mg/kg), phenytoin (100 mg/kg) and phenobarbital (65 mg/kg), and 0.9% sodium chloride (saline control) were given orally by gavage as aqueous solutions on each of five successive mornings. Nine hours after the fifth dose all animals received a single (acute challenge) dose of a different anticonvulsant drug, sodium valproate (300 mg/kg) or phenytoin (100 mg/kg), or saline. Animals were killed 16 hr after the acute dose.

Heparinized blood was collected when the animals were killed, and plasma was analyzed for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total protein (Worthington Kits) using a Roto-chem CFA 2000 centrifugal analyzer, and ornithine carbamyl transferase [5]. For histopathologic evaluation, liver tissue was fixed in formalin, embedded in paraffin, and sections were stained with hematoxylin and eosin or periodic acid-Schiff. The remaining liver was homogenized in 0.25 M sucrose (pH 7.4). Reduced glutathione (sulfosalicylic acid non-precipitable reduced sulfhydryls) concentration was determined in the liver homogenate [6]. Microsomes prepared with calcium chloride [7] were assayed for: cytochrome(s) P-450 [8], aniline hydroxylase [9], aminopyrine demethylase [9], glucose-6-phosphatase [10], and protein [11]. Enzyme activities are expressed as nmoles or μmoles of product formed per mg of microsomal protein per min.

The data were analyzed by two-way analysis of variance (ANOVA). Significance ($P < 0.05$) was determined for each of the subacute and acute treatment regimens, as well as their potential interaction, for each variable measured. Statements of significance with respect to a particular drug and regimen are made in reference to control (saline) animals given the same regimen.

Results and discussion

The rationale for our approach in setting up potential hepatotoxic interactions between phenytoin, sodium valproate, and phenobarbital was based on two factors. First, both phenytoin and valproic acid are converted in the liver by the cytochrome(s) P-450-linked mixed-function oxidase system to potentially reactive metabolites [3, 12–14]. Such metabolites could trigger injury either directly, in the case of valproate, or indirectly, in the case of phenytoin by acting as a hapten leading to secondary immune responses [12] or by causing mild subclinical hepatotoxicity upon which a superimposed hypersensitivity reaction leads to overt liver damage [1]. Second, there are many ways in which combinations of drugs may cause or enhance a toxic response. Damage may result from direct interaction at the cellular targets of toxicity or may be secondary to pharmacokinetic interactions. The latter interactions between anticonvulsant drugs include induction of oxi-

dative metabolism, displacement from plasma protein binding sites, and competitive or non-competitive inhibition of metabolism [15, 16]. Inductive interactions are of interest with respect to anticonvulsant-induced hepatotoxicity because the mixed-function oxidase system is inducible by anticonvulsant drugs such as phenobarbital and phenytoin [17, 18] which may thereby increase the formation of a reactive metabolite of a concomitantly administered drug.

The results of hepatic assays (glutathione, microsomal protein, cytochrome P-450, aniline hydroxylase, aminopyrine demethylase, and glucose-6-phosphatase) are shown in Fig. 1, and the statistically significant changes and interactions are indicated in Table 1. Phenytoin or phenobarbital administered as a subacute regimen of five daily doses at approximately ten times human therapeutic dose levels, induced mixed-function oxidase in the liver as manifested by increased cytochrome P-450 levels, aniline hydroxylase activity, and aminopyrine demethylase activity. Microsomal protein was also increased in phenobarbital, but not phenytoin, pretreated animals.

The induction of hepatic mixed-function oxidase by phenytoin in the present study is consistent with the results of other repeated dose experimental studies [18–21]. Collectively these findings indicate that phenytoin is a moderate inducer of cytochrome P-450-linked mixed-function oxidase and potentially capable of affecting the metabolism of many drugs that undergo oxidative metabolism in the liver. The present study also indicates that even a single high dose of phenytoin can induce mixed-function oxidase in rat liver. The statistically significant interaction between the subacute phenobarbital regimen and acute phenytoin with respect to cytochrome P-450 level (Table 1) is a reflection that acute phenytoin administration did not cause additional induction in phenobarbital-pretreated animals. The subacute phenobarbital and phenytoin regimens increased hepatic glutathione concentration, a phenomenon which may be related to the induction of drug metabolism.

In the present study, one or five successive daily doses of 300 mg/kg sodium valproate did not affect cytochrome P-450, aniline hydroxylase, or aminopyrine demethylase, providing additional experimental evidence that valproate is not an inducer of mixed-function oxidase. Earlier reports in rats indicated that sodium valproate at lower doses does not affect cytochrome P-450, cytochrome b_5, or aniline hydroxylase activity [22, 23]. Sodium valproate does not appear to induce mixed-function oxidase in human beings as determined from antipyrine half-life and glucaric acid excretion tests [24, 25].

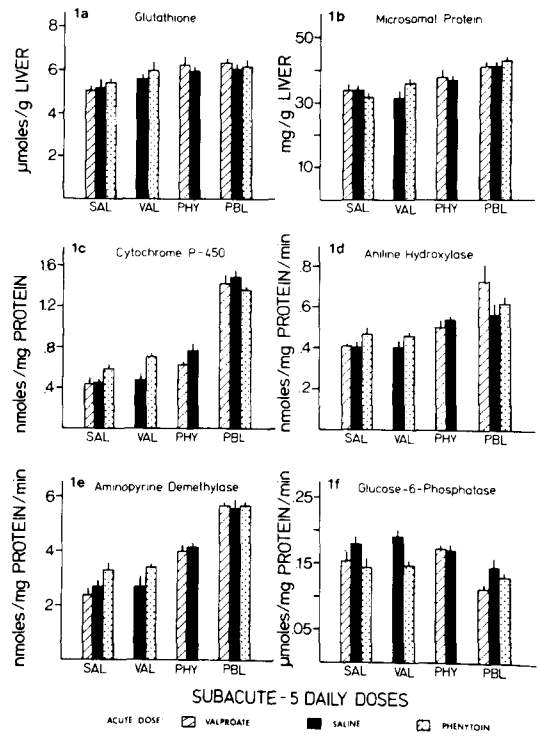

Fig. 1. Hepatic variables after subacute saline (SAL, control), sodium valproate (VAL, 300 mg/kg), phenytoin (PHY, 100 mg/kg), or phenobarbital (PBL, 65 mg/kg), followed 9 hr later by acute saline (control), sodium valproate (300 mg/kg), or phenytoin (100 mg/kg) administration, with sacrifice 16 hr after the acute dose: (1a) glutathione, (1b) microsomal protein, (1c) cytochrome P-450, (1d) aniline hydroxylase, (1e) aminopyrine demethylase, (1f) glucose-6-phosphatase. Bars represent the mean ± SEM of four rats. Statistically significant changes are indicated in Table 1.

Table 1. Statistically significant changes determined by two-way ANOVA in hepatic variables from Fig. 1

	Treatment regimen*			
Variable	Subacute phenobarbital (5 daily doses)	Subacute phenytoin (5 daily doses)	Acute phenytoin (saline or valproate pretreatment)	Acute valproate (saline or phenobarbital pretreatment)
Glutathione	↑ ($P < 0.01$)	↑ ($P < 0.025$)		
Microsomal protein	↑ ($P < 0.003$)			
Cytochrome P-450	↑ ($P < 0.001$)	↑ ($P < 0.001$)	↑ ($P < 0.005$)	
Aniline hydroxylase	↑ ($P < 0.001$)	↑ ($P < 0.001$)	↑ ($P < 0.05$)	
Aminopyrine demethylase	↑ ($P < 0.001$)	↑ ($P < 0.001$)	↑ ($P < 0.025$)	
Glucose-6-phosphatase	↓ ($P < 0.01$)		↓ ($P < 0.01$)	↓ ($P < 0.05$)

Interactions between: (1) subacute phenobarbital regimen and acute phenytoin with respect to cytochrome P-450 ($P < 0.05$); and (2) subacute valproate regimen and acute phenytoin with respect to microsomal protein ($P < 0.05$).

* There were no significant changes attributable to subacute valproate treatment by itself.

Despite mixed-function oxidase induction in the liver, combinations of anti-convulsant drugs did not elicit hepatotoxicity in this experimental model, based on the absence of histopathologic changes, lack of toxicologically significant increases in plasma enzyme markers of hepatotoxicity (data not shown), and lack of significant decreases in hepatic mircosomal assays. The only hepatic variable that showed consistent treatment-related decreases was glucose-6-phosphatase, a microsomal enzyme which has been used as a marker for xenobiotic-induced hepatic damage, particularly to the endoplasmic reticulum [5]. However, the reduction of glucose-6-phosphatase activity in phenobarbital-pretreated animals likely reflects, at least in part, a dilution of microsomal enzyme activity by increased protein within the induced liver. The reduction of glucose-6-phosphatase activity after an acute dose of phenytoin was not apparent after the five successive daily doses of phenytoin. The decrease in glucose-6-phosphatase activity after acute sodium valproate did not correlate with decreases in other hepatic microsomal variables and is not considered to be a manifestation of significant toxicity.

In summary, these experiments indicate that even at high doses, which induce mixed-function oxidase activity, the anticonvulsant drugs phenytoin, phenobarbital, and sodium valproate do not interact to cause hepatotoxicity in rats. If these results are predictive for man, combinations of anticonvulsant drugs are unlikely by themselves to cause hepatotoxicity. While anticonvulsant polytherapy may increase the risk of hepatotoxicity, other factors, such as an idiosyncratic impairment of reactive metabolite detoxification, are probably equally important in the development of liver injury in human beings.

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